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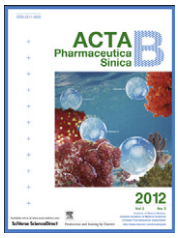

ORIGINAL ARTICLE

Microscopic identification of the remnant hair or feather of five animal drug components in Shenrongbian pill

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KEY WORDS

Penis et Testis Canis;
Penis et Testis Equi;
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Penis et Testis Mustelae;
Musculus et Bonis Passeris;
Animal drugs;
Remnant hair;
Microscopic identification

Abstract A comparative study was performed to identify the microscopic characteristics of hair or feather in the five animal drug components contained in Shenrongbian pill. Penis et Testis Canis is 40 ± 0.07 in the medulla index, with long circular, banana or triangular circular shaped medulla cells arranged in one line or network, and the hair cuticle is in imbrication (d, m) and flat wave (p) shape. Penis et Testis Equi is 66 ± 0.10 in the medulla index, with ellipse, spindle or long strip-shaped medulla cells arranged in network, and the hair cuticle is in flat wave shape. Penis et Testis Bovis is 67 ± 0.05 in the medulla index, with rectangle, spindle or polygon-shaped medulla cells arranged in ladder or network form, and the hair cuticle is in flat wave shape. Penis et Testis Mustelae is 29 ± 0.05 in the medulla index, with ellipse-like, square-like or circular shaped medulla cells arranged in one line generally, and the hair cuticle is in acuminate (d, m), imbrication (m,p) and slightly flat wave (p) shape. Musculus et Bonis Passeris is 24 ± 0.05 in the medulla index, with bamboo joint-shaped barbs and unclear medulla cells, without hair cuticle.

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1. Introduction

Shenrongbian pill is a commonly used traditional Chinese patent medicine with the goal of preventing and curing sexual failure for health protection use. The authors of this paper have previously reported the microscopic identification of Shenrongbian pill components[1]. It contains five animal drug components: Penis et Testis Canis, Penis et Testis Equi, Penis et Testis Bovis, Penis et Testis Mustelae and Musculus et Bonis Passeris. There are rare reports on the microscopic structure of the remnant hair or feather of the five animal components, including the hair on the back of *Canis familiaris* Linnaeus, *Bos taurus domesticus* Gmelin, *Equus caballus orientalis* Noack and *Martes zibellina*[2]. Previous studies have shown that it is possible to identify animal drug components by the microscopic characteristics of the remnant hair[3–7]. In this study, we used a microscopic identification method[8] to characterize the tissue morphology of the remnant hair or feather from the five animal components that make up Shenrongbian pill.

2. Materials and method

2.1. Materials and apparatus

The five animal component drugs, Penis et Testis Canis, Penis et Testis Equi, Penis et Testis Bovis, Penis et Testis Mustelae and Musculus et Bonis Passeris were supplied by Dalian Pharmaceutical Factory of T.C.M. and Shenyang Institute For Drug Control, respectively. All the specimens were authenticated by Professor Tingguo Kang and deposited at College of Chinese Materia Medica of Liaoning University of T.C.M. in China. The details are shown in Table 1, and the photos are in Fig. 1.

Chloral hydrate was prepared according to procedures described in Appendix 100, Pharmacopoeia of the People's Republic of China, 2000; dilute glycerin was prepared according to procedures described in Appendix 102, Pharmacopoeia of the People's Republic of China, 2000.

Microscopy was performed using an Olympus-BX50 system biologic microscope equipped with an Olympus-DP10 camera and processed with a free software Win-measure (Stephen M. Younts).

2.2. Method

The remnant hair or feather scraped from the surface of the drug components was washed 1–3 times by chloral hydrate and then sealed with dilute glycerin. Each sample was observed under the microscope. Representative dimensions with respect to diameter of hair or feather were chosen to be measured by Win-measure for differentiation. The sump way[9] was used to observe the shape of hair cuticle.

3. Results

3.1. Penis et Testis Canis

The remnant hair is light yellow or brownish yellow color, 56.00 ± 14.14 μm in diameter, 22.10 ± 5.82 μm in medulla diameter and 40 ± 0.07 in medulla index (diameter of medulla/diameter of hair shaft × 100%). The hair root is connected to the enlarged hair follicle. The hair shaft is very long, with even thickness, while the tip of hair gradually becomes acuate. The medulla cell is long circular, banana or triangular circular-like shape, arranged in 1 line generally or in net form (2–3 lines) with gray or brown dark pigment granules and a dimly visible outline. The pigment granules fill the whole medulla in some instances. The hair cortex is relatively broad. The hair cuticle wrinkle is imbricate-shaped in the third part to the distal end (D) and the middle part (M), and gradually changes to a flat wave shape in the third part to the proximal end (P), 18.40 ± 5.48 μm in vertical length in M (Fig. 2(A)).

3.2. Penis et Testis Equi

The remnant hair is reddish brown or brownish yellow, 61.40 ± 12.62 μm in diameter, 40.70 ± 11.93 μm in medulla diameter and 66 ± 0.10 in medulla index. The medulla cell is ellipse, spindle or long strip-shaped, arranged in net-like or unclear format with dark brown or gray pigment granules filling the whole medulla, but rarely arranged in one single line. The hair cortex is relatively narrow. The hair cuticle wrinkle is flat wave-shaped, 10.40 ± 4.53 μm in vertical length in M (Fig. 2(B)).

Table 1 Origins of commercial crude drugs of the five animal drugs.

Sample	Original	Sample number	Supplier	Locality
Penis et Testis Canis (A)	*Canis familiaris* Linnaeus	*P.T.C.*I	[a]	1
		*P.T.C.*II	[b]	2
Penis et Testis Equi (B)	*Equus asinus* Linnaeus	*P.M.A.*I	[a]	1
		*P.M.A.*II	[b]	2
Penis et Testis Bovis (C)	*Bos taurus domesticus* Gmelin	*P.M.B.*I	[a]	1
		*P.M.B.*II	[b]	2
Penis et Testis Mustelae (D)	*Mustela vison* Schreber	*P.T.M.*I	[a]	3
		*P.T.M.*II	[b]	4
Musculus et Bonis Passeris	*Passer montanus* Linnaeus	*M.B.P.*I	[a]	1
		*M.B.P.*II	[b]	3

1: Dalian, Liaoning, China; 2: Shenyang, Liaoning, China; 3: Inner Mongolia, China; 4: Shanxi, China.
[a]Dalian Pharmaceutical Factory of T.C.M.
[b]Shenyang Institute for Drug Control.

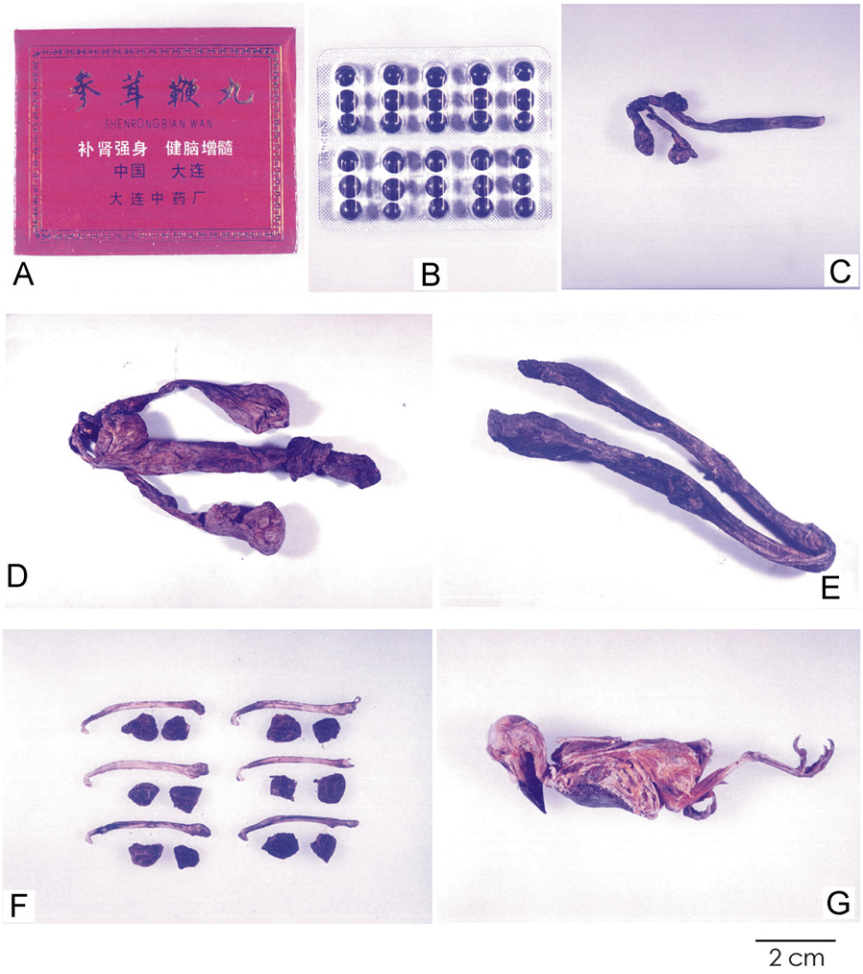

Figure 1 The patent medicine and the five animal drugs of Shenrongbian pill. (A) External package of Shenrongbian pill; (B) internal package of Shenrongbian pill; (C) Penis et Testis Canis; (D) Penis et Testis Equi; (E) Penis et Testis Bovis; (F) Penis et Testis Mustelae; (G) Musculus et Bonis Passeris.

3.3. *Penis et Testis Bovis*

The remnant hair is light yellow or yellow–brown, a little thicker in the middle part, 82.90±35.82 μm in diameter, 59.10±33.23 μm in medulla diameter and 67±0.13 in medulla index. The medulla cell is rectangular, spindle- or polygon-like, arranged in a ladder-like shape made up of 1–5 lines of rectangular cells or net-like, with dark gray or brown pigment granules and a dimly visible outline. The pigment granules fill the whole medulla in some instances. The hair cortex is relatively narrow. The hair cuticle wrinkle is in a flat wave shape, with 10.80±5.57 μm vertical length in M (Fig. 2(C)).

3.4. *Penis et Testis Mustelae*

The remnant hair is yellow–brown or red–brown, much thicker in the middle part and the third part to the proximal end, 23.60±16.41 μm in diameter, 7.50±7.26 μm in medulla diameter and 29±0.05 in medulla index in the middle part. The medulla cell is an ellipse, square, or circular shape, arranged in 1 line mostly or 2–3 lines occasionally, filled by dark brown or brownish-red pigment granules. There are brownish-red pigment granules or stripes in the hair cortex. The hair cuticle wrinkle is acuminate in shape in D and M, and becomes imbricate gradually in M and P, and becomes slightly flat-wave shaped in P gradually, with vertical length of 23.20±5.59 μm in M (Fig. 2(D)).

3.5. *Musculus et Bonis Passeris*

The remnant feather is light yellow or almost colorless, with a comparatively thicker and hollow quill. There are aftershafts on the both sides of the shaft, and many thin and long barbs in the aftershaft. The developed medulla cell of the shaft and thicker aftershaft is polygon-like, rectangular or square. The barb is bamboo joint-like in shape, with 128.20±8.92 μm in length joint, 8.20±2.15 μm in diameter. The medulla is narrow, linear in arrangement or obscure in appearance, 2.00±0.74 μm in diameter, 24±0.05 in medulla index. The medulla cell is indistinct. There are dark brown or reddish-brown pigment granules in the joints (Fig. 3).

4. Discussion

The remnant feather of Musculus et Bonis Passeris has different microscopic characteristics from the other four animal

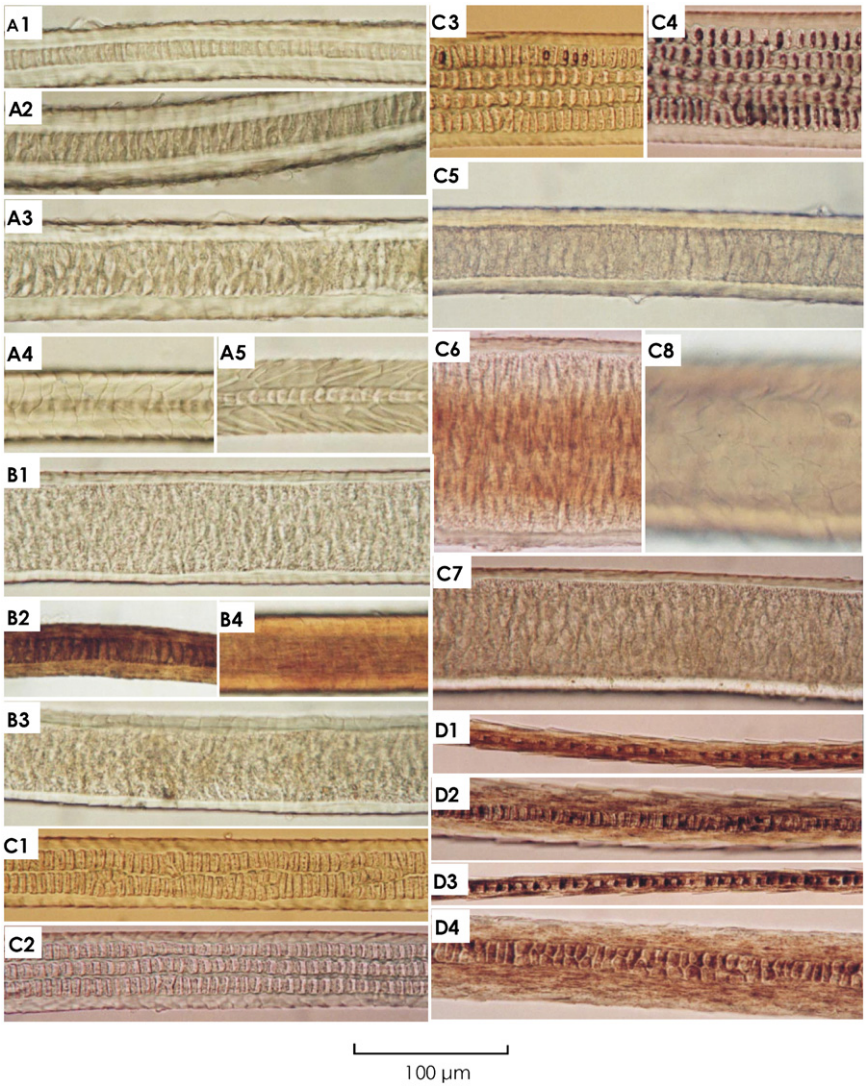

Figure 2 Microscopic features of remnant hair of four penis crude drugs. (A) Penis et Testis Canis: 1, cell of medulla – single line; 2, cell of medulla – similar netlike; 3, medulla full of pigment granule; 4 and 5, hair cuticle; (B) Penis et Testis Equi: 1 and 2, cell of medulla – netlike; 3, medulla full of pigment granule; 4, hair cuticle; (C) Penis et Testis Bovis: 1–4, cell of medulla – ladderlike; 5 and 6, cell of medulla – netlike; 7, medulla full of pigment granule; 8, hair cuticle; (D) Penis et Testis Mustelae: 1–3, cell of medulla – single line; 4, cell of medulla – 2 lines.

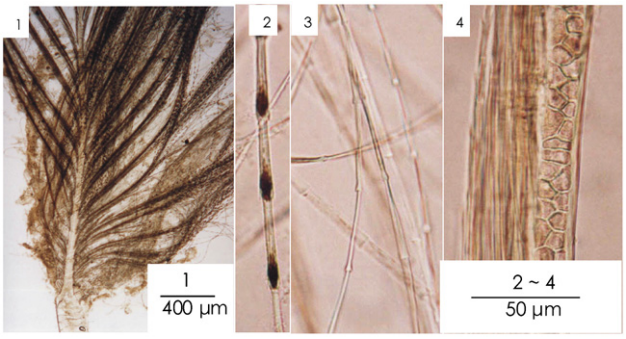

Figure 3 Microscopic features of remnant feather of Musculus et Bonis Passeris. 1, a spray of feather; 2 and 3, barbs; 4, shaft (cell of medulla).

components. The remnant hair of the other four animal components differ from each other in size, color, shape of the medulla cell, the medulla index, and the shape of hair cuticle (Tables 2 and 3).

The main microscopic features of the remnant hair of Penis et Testis Canis and Penis etTestis Bovis are consistent with those of hair on the back of *Canis familiaris* L. and *Bos taurus domesticus* Gmelin as reported (though the number of the samples in this study is 10, while in previous report is 5), in spite of the rarely reported longitudinal morphology of medulla cells[2]. The diameter of the hair on the back of *Canis familiaris* L. is 60–120 μm, less than that of Penis et Testis Canis (56.00 ± 14.14 μm (40–80)). The medulla index of the hair on the back of *Canis familiaris* L. and *Bos taurus domesticus* Gmelin is 40–60, a little higher than that of Penis et Testis Canis (40 ± 0.07 (33–55)) and a little lower

Table 2 Comparative differentiation of the microscopic features of remnant hair (or feather) from the five animal drugs (μm).

Item	Penis et Testis Canis	Penis et Testis Equi	Penis et Testis Bovis	Penis et Testis Mustelae	Musculus et Bonis Passeris (barbs of feather)
Shape	Enlarged hair follicle, long hair shaft with even thickness, gradually acute hair tip	Nearly the same as the left	A little thicker in the M[a]	Much thicker on the M and P[a]	Similar bamboo joint, thin and long
Color	Light yellow or brownish yellow	Reddish brown or brownish yellow	Light yellow or yellowish brown	Yellowish brown or reddish brown	Light yellow or near colorless
Diameter (M)					
Hair shaft	56.00 ± 14.14(40–80)	61.40 ± 12.62 (40–100)	82.90 ± 35.82 (40–120)	23.60 ± 16.41 (16–70)	8.20 ± 2.15(6–12) [c]
Medulla	22.10 ± 5.82	40.70 ± 11.93	59.10 ± 33.23	7.50 ± 7.26	2.00 ± 0.74[c]
Medulla index[b]	40 ± 0.07(33–55)	66 ± 0.10(49–77)	67 ± 0.13(38–87)	29 ± 0.05(25–40)	24 ± 0.05(17–34)[c]
Cell of medulla					
Shape	Long circular, banana or triangular circular-like	Similar ellipse, spindle or long strip	Rectangle, spindle or polygon-like	Similar ellipse, similar square or circular	Unclear [d]
Form of arrangement	1 line or netlike (2–3 lines)	Netlike or unclear (more), 1 line (a few)	Ladder like (1–5 lines) or netlike	1 line (more), 2–3 lines (a few)	
Pigment granule	Grayish dark or brownish dark	Brownish dark or grayish dark	Grayish dark or brownish dark	Brownish dark or brownish red	Brownish dark or reddish brown
Cortex	Wide	Narrow	Narrow	Wide	Wide
Hair cuticle					–[e]
Shape	Imbrication (D[a], M), flat wave (P)	Flat wave	Flat wave	Acuminate (D, M), imbrications (M, P), slightly flat wave (P)	
Vertical length of (M)	18.40 ± 5.48	10.40 ± 4.53	10.80 ± 5.57	23.20 ± 5.59	

$N = 10$.
[a]D, the third part to the distal end; M, the middle part; P, the third part to the proximal end.
[b]Medulla index = diameter of medulla/diameter of hair shaft × 100%.
[c]Part of joint of barbs.
[d]Cell of medulla of shaft and thicker aftershaft is polygon-like, rectangle or square in shape.
[e]Not applicable.

Table 3 Index of microscopic identification of remnant hair (feather) of the five animal drugs.

Index	Animal drug
1. Hair shaft is long cylinder, with gradually acute hair tip, presence of hair cuticle	
2. Medulla index < 50, 2–3 shapes of the wrinkle of hair cuticle	
3. Hair shaft is light yellow or brownish yellow in color, 56.00 ± 14.14 μm in diameter of the middle part, 40 ± 0.07 in medulla index. The cells of medulla arrange in 1 line mostly. The wrinkles of hair cuticle are imbrication in (D, M), flat wave gradually in (P)	Penis et Testis Canis
3. Hair shaft is yellowish brown or reddish brown in color, 23.60 ± 16.41 μm in diameter of the middle part, 29 ± 0.05 in medulla index. The cells of medulla arrange in 1 line mostly. The wrinkles of hair cuticle are acuminate(D, M), imbrication(M, P), slightly flat wave(P)	Penis et Testis Mustelae
2. Medulla index > 50, the shape of the wrinkle of hair cuticle is flat wave	
4. Hair shaft is light yellow or yellowish brown in color, 82.90 ± 35.82 μm in diameter of the middle part, 67 ± 0.13 in medulla index. The cell of medulla is rectangle, spindle or polygon-like arranging ladderlikely or netlikely	Penis et Testis Bovis
4. Hair shaft is reddish brown or brownish yellow in color, 61.40 ± 12.62 μm in diameter of the middle part, 66 ± 0.10 in medulla index. The cell of medulla is similar ellipse, spindle or long strip arranging netlikely or unclearly	Penis et Testis Equi
1. Hair shaft of rarbs is similar bamboo joint, 8.20 ± 2.15 μm in diameter of joint, presence of brownish dark or reddish brown pigment granule, 24 ± 0.05 in medulla index, 128.20 ± 8.92 μm in length of a part of joint, absence of hair cuticle	Musculus et Bonis Passeris

than that of Penis et Testis Bovis (67 ± 0.13 (38–87)). The hair cuticle wrinkle from D of the hair on the back of *Bos taurus domesticus* Gmelin is flat while that of Penis et Testis Bovis is flat wave-shaped.

The above observations indicate that the hair of different parts from the same species is basically consistent in their main features and possess stable biological features, even if in patent medicines[1]; however, the hair's morphological features are dissimilar between species. Thus, the conclusion may be reached that it is scientifically feasible to identify the animal drug components of the patent medicines containing them based on the microscopic characteristics[8] of their remnant hair or feather.

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